Summary of Ph.D. thesis

SYNTHESIS OF STERANE-CONDENSED NITROGEN-CONTAINING HETEROCYCLES FROM BIFUNCTIONAL SEX HORMONE DERIVATIVES

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1. Introduction and aims

Steroids with a four-ring framework are natural compounds found in living organisms. Their biological activity depends largely on the quality, position and spacial orientation of functional groups on the sterane core and on the stereostructure of the whole molecule. Today, one of the main driving force of steroid research is the production of semi-synthetic sex hormone derivatives which, as a result of modifications, lead to the reduction or elimination of the primary hormonal effect with the simultaneous appearance of a new, completely different bioactivity. Many previously synthesized heterocyclic steroidal compounds have been shown to inhibit the proliferation of tumor cells of diverse origins or have direct cytotoxic activity, therefore such modifications of sex hormones was one of the objectives of the present study.

Based on the literature background and the synthetic results of the Steroid Chemistry Research Group, our aim was to produce new nitrogen-containing heterocyclic derivatives fused to the sterane skeleton. We designed the feasibility and the optimization of the conditions of each reaction on rings A and D of sex hormones. We also wanted to investigate the effect of substituents of the reagents on the ring-closure processes. Our aim was to determine the exact structure of the compounds and to subject all derivatives to *in vitro* pharmacological examinations in cooperation.

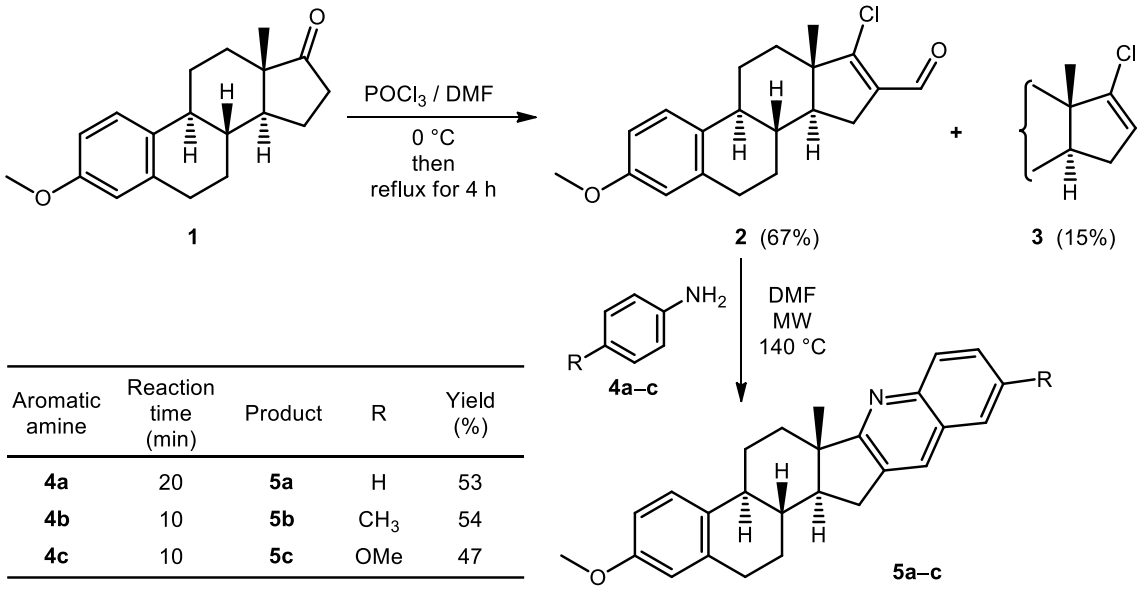
2. Experimental methods

All of the reactions were performed in millimolar scale, and were monitored by thin layer chromatography (TLC). In addition to conventional heating, in some cases microwave (MW) irradiated reactions were also carried out in closed vessels. The crude products were purified by flash cromatography. The structure of each product was determined by different analytical methods (ESI-MS, IR, NMR).

3. Scientific results

3.1. Steroidal β-chlorovinyl aldehyde (**2**) was synthesized from estrone-3-methylether (**1**) by the *Vilsmeier-Haack* reaction ($POCl_3$, DMF). The cyclization of the bifunctional steroid (**2**) with aniline (**4a**) under solvent-free conditions or in DMF afforded the desired sterane-fused quinoline (**5a**) in moderate yield (*Scheme 1*).

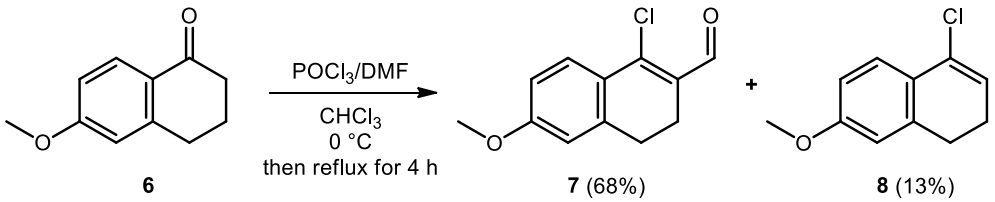
3.2. Similar transformations with *p*-toluidine (**4b**) and *p*-anisidine (**4c**) led to the heterocyclic products (**5b**, **5c**) in moderate yields, while reactions failed with aniline derivatives containing electron-withdrawing groups (EWG) (Cl, NO_2). Only certain intermediates, which underwent decomposition during the purification process, could be detected by TLC in these latter cases.



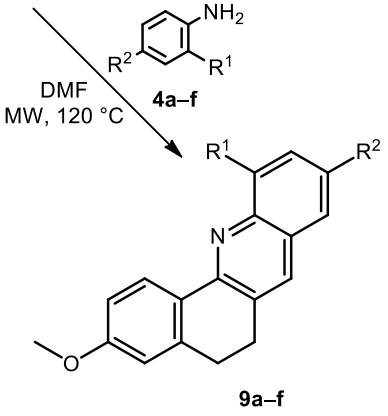
Aromatic amine	Reaction time (min)	Product	R	Yield (%)
4a	20	**5a**	H	53
4b	10	**5b**	CH_3	54
4c	10	**5c**	OMe	47

Scheme 1

3.3. For further experiments, 6-methoxytetralone (**6**) was used as a model of ring A of the sterane core. Non-steroidal β-chlorovinyl aldehyde (**7**) was prepared with the *Vilsmeier-Haack* complex, similarly to the synthesis of **2**. The microwave-assisted reactions of **7** with aniline (**4a**) and substituted anilines (**4b–f**) in DMF resulted in benz[*c*]acridine derivatives (**9a–f**) in varying yields, depending on the steric and electronic character of the substituents on the aromatic moiety (*Scheme 2*).



Aromatic amine	Reaction time (min)	Product	R^1	R^2	Yield (%)
4a	5	**9a**	H	H	76
4b	2	**9b**	H	CH_3	90
4c	2	**9c**	H	OMe	89
4d	20	**9d**	Cl	H	35
4e	10	**9e**	H	Cl	54
4f	10	**9f**	H	Br	62

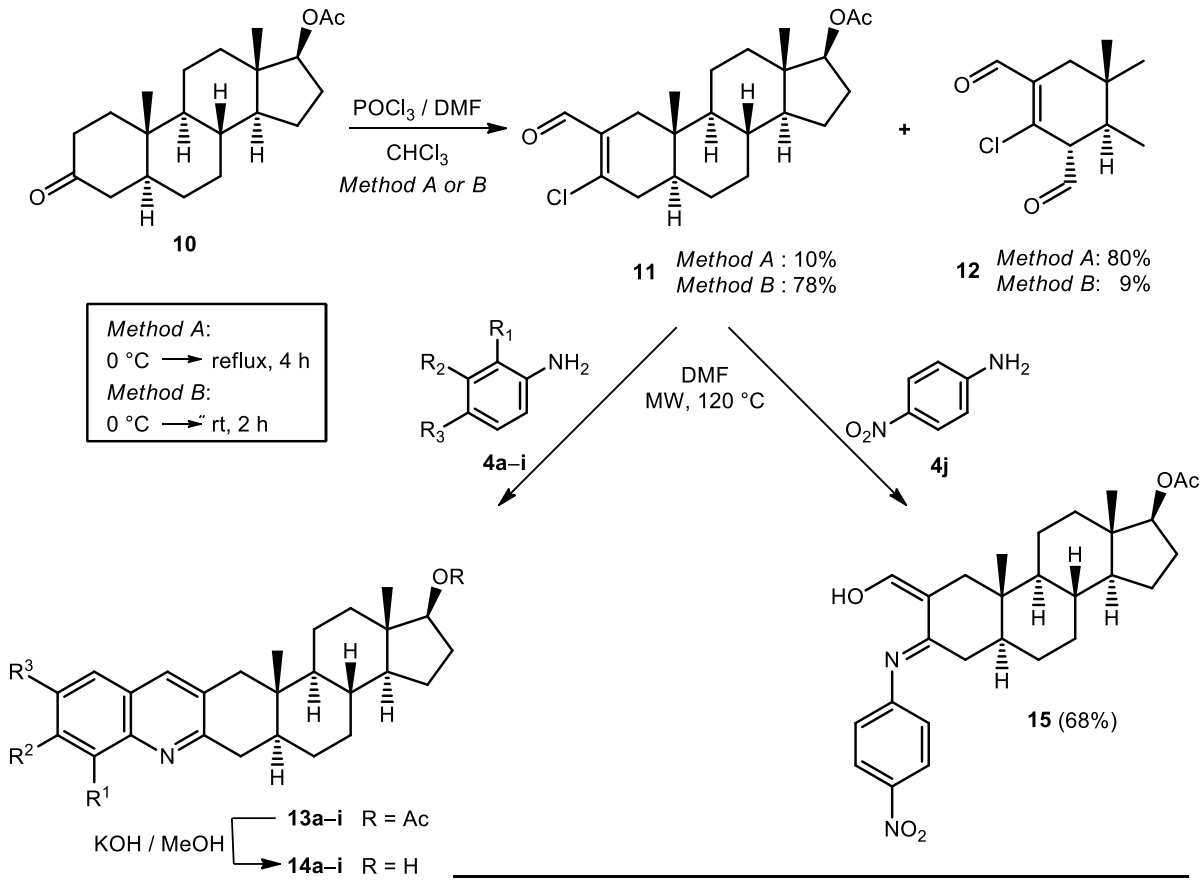


Scheme 2

3.4. The microwave-assisted reaction of β-chlorovinyl aldehyde (**11**) (synthesized from 17β-acetoxy-5α-dihydrotestosterone (**10**) by the *Vilsmeier-Haack* reaction at room temperature) with aniline (**4a**) or substituted aromatic amines (**4b–i**) in DMF afforded ring A-fused quinolines (**13a–i, 13g', 13i'**). *Ortho*-substituted amines (**4d**, **4h**) lowered the yields of the desired products (**13d**, **13h**), while syntheses with *meta*-substituted aniline derivatives (**4g**, **4i**) resulted in regioisomeric mixtures (**13g** and **13g'** or **13i** and **13i'**). Electronic effect of the substituents was also observed during the reactions. Electron-donating groups on the reagents increased, while electron-withdrawing groups decreased the maximum amount of the heterocyclic compounds (*Scheme 3*).

3.5. During the reaction of **11** with 4-nitroaniline (**4j**) the formation of steroidal quinoline was not observed, a *N*-aryl-imino-enol product (**15**) was isolated instead.

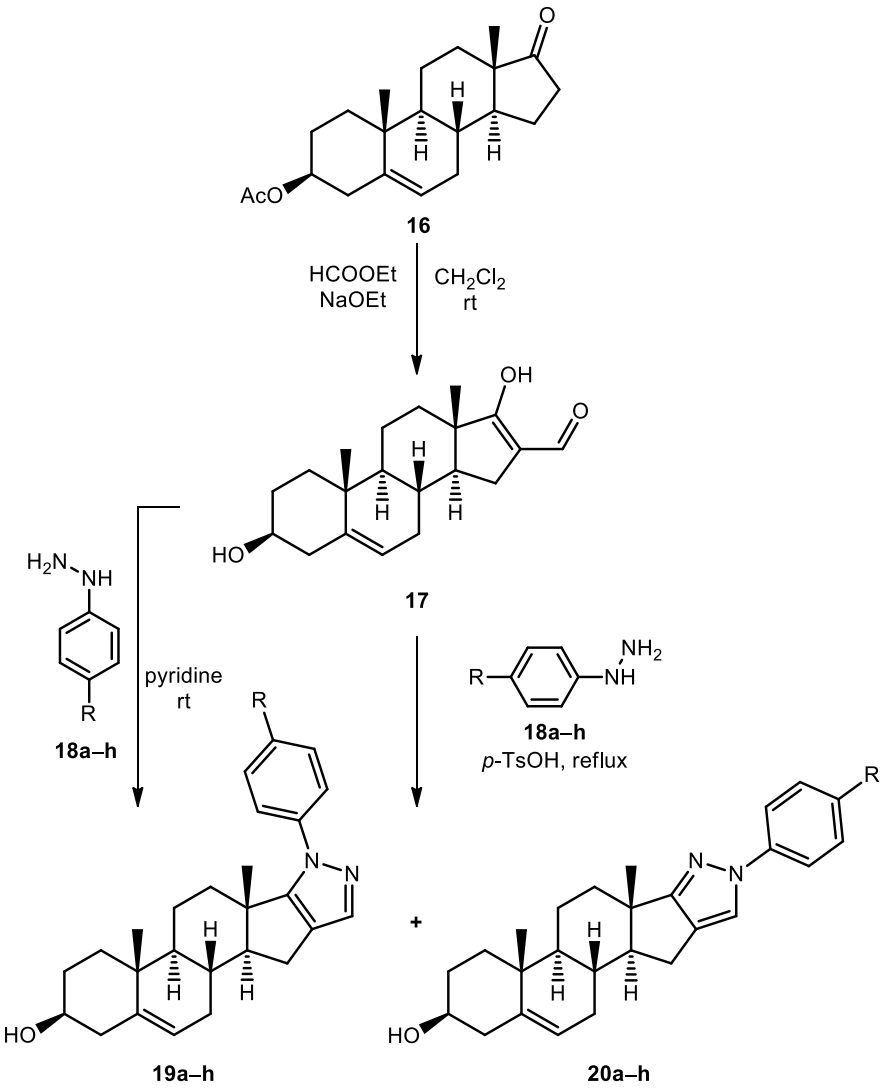
3.6. The ring A-fused products (**13a–i, 13g'**) were deacetylated in alkaline (KOH) methanol to afford the corresponding 17β-OH analogues (**14a–i, 14g'**).



Aromatic amine	R^1	R^2	R^3	Reaction time (min)	Product(s)	Yield(s) (%)
4a	H	H	H	5	**13a**	76
4b	H	H	CH_3	2	**13b**	91
4c	H	H	OMe	2	**13c**	92
4d	Cl	H	H	20	**13d**	43
4e	H	H	Cl	10	**13e**	59
4f	H	H	Br	10	**13f**	60
4g	H	Cl	H	10	**13g** + **13g'**	46 + 16
4h	CH_3	H	H	5	**13h**	73
4i	H	CH_3	H	2	**13i** + **13i'**	90

Scheme 3

3.7. 16-hydroxymethylene-dehydroepiandrosterone (**17**) was next synthesized from dehydroepiandrosterone-3β-acetate (**16**) by the *Claisen* condensation. Compound **17** was treated with phenylhydrazine (**18a**) under general *Knorr* conditions in ethanol in the presence or absence of acetic acid catalyst leading to the regioselective formation of a single arylpyrazole (**19a**). Reaction of **17** with phenylhydrazine hydrochloride (**18a**·HCl) in pyridine also proved to be regioselective (**19a**), while a regioisomeric mixture of **19a** and **20a** in a ratio of 1:1 was obtained in refluxing ethanol in the presence of 0.3 equivalent amount of *p*-TsOH (*Scheme 4*).



Reagent	R	Regioisomeric ratio **19** : **20**	Yield (%)
18a	H	1 : 1	85
18b	CH_3	1 : 2	87
18c	OMe	1 : 2	80
18d	F	2 : 1	83
18e	Cl	2 : 1	82
18f	Br	2 : 1	85
18g	CN	10 : 1	82
18h	NO_2	1 : 0	84

Scheme 4

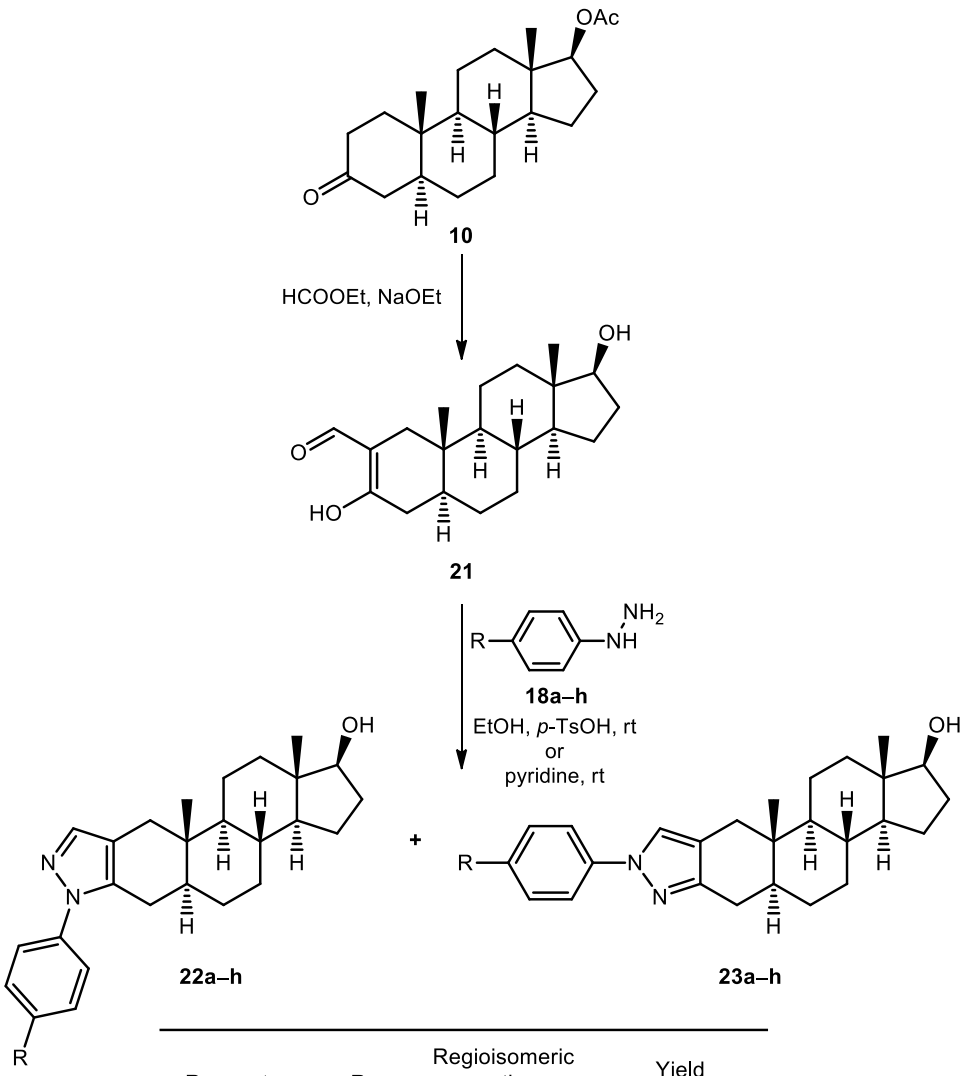
3.8. Catalytic reactions (0.3 equiv. *p*-TsOH) of **17** with *p*-substituted phenylhydrazine hydrochloride salts (**18b–h**·HCl) in ethanol afforded the regioisomeric mixture of the products (**19b–g** and **20b–g**) in different ratios, with the only exception of that with *p*-nitro-phenylhydrazine (**18h**) where the synthesis was regioselective (only product **19h**). The ratio of

the regioisomeric pairs was shifted towards the formation of **19** over **20** in case of EWGs and **20** over **19** in case of EDGs (*Scheme 4*).

3.9. 2-Hydroxymethylene-5α-dihydrotestosterone (**21**) was also prepared from 17β-acetoxy-5α-dihydrotestosterone (**10**) by *Claisen* condensation. The reaction of **21** with phenylhydrazine hydrochloride (**18a**) or *p*-substituted phenylhydrazine derivatives (**18b–h**·HCl) in acidic ethanol (0.3 equiv. *p*-TsOH) at room temperature led to the two possible regioisomers (**22a–h** and **23a–h**) (*Scheme 5*).

3.10. Transformations of the bifunctional steroid derivative (**21**) with arylhydrazine hydrochloride salts (**18a–h**·HCl) in pyridine at room temperature also resulted in the mixtures of regioisomers (**22a–h** and **23a–h),** in contrast to the similar reactions on ring D of the sterane core. The major formation of **23** over **22** in case of strong EWG-containing (CN, NO_2) reagents (**18g**, **18h**) was observed. EDGs on the hydrazines did not have any significant impact on the isomeric distribution.

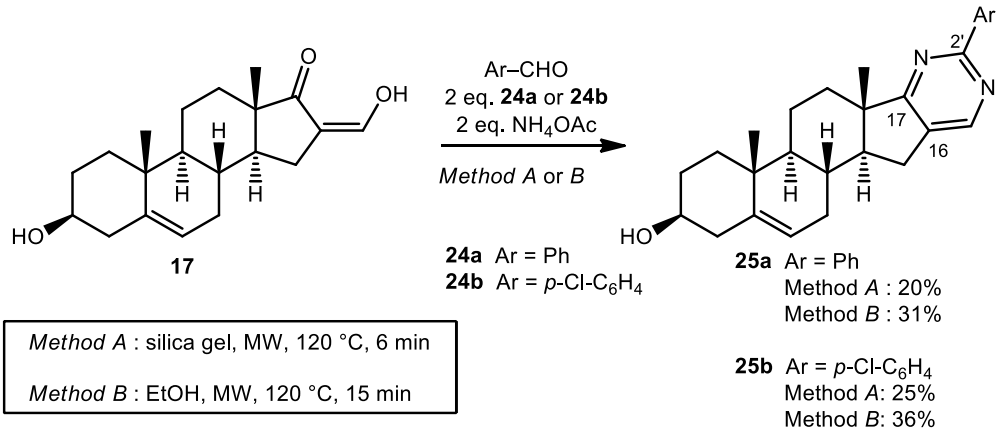
3.11. According to the observations, the formation of ring A-fused pyrazoles followed a different reaction mechanism than that of the ring D-condensed analogues. In the latter case, the formation of hydrazone intermediates followed by intramolecular cyclocondensation occurs, while two consecutive dehydration steps of the dihydroxypirazolidine intermediates may result in the ring A-fused pyrazole derivatives.



Reagent	R	Regioisomeric ratio **22** : **23**	Yield (%)
18a	H	4 : 3	89
18b	CH_3	1 : 1	82
18c	OMe	1 : 1	82
18d	F	4 : 3	79
18e	Cl	4 : 3	85
18f	Br	4 : 3	84
18g	CN	1 : 2	78
18h	NO_2	1 : 2	84

Scheme 5

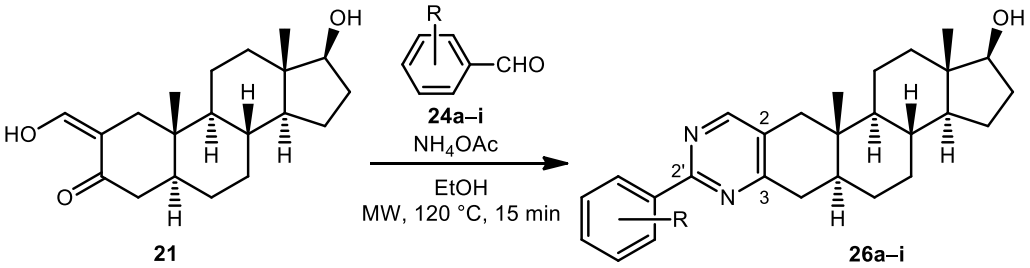
3.12. The silica gel-supported, solvent-free, microwave-assisted multicomponent reaction of 16-hydroxymethyene-dehydroepiandrosterone (**17**), ammonium acetate and benzaldehyde (**24a**), or *p*-Cl-benzaldehyde (**24b**) (1:2:2 molar ratio) resulted in steroidal ring D-fused arylpyrimidine derivatives (**25a**, **25b**) in sluggish yields (20–25%). When the reactions were repeated using the same reactant ratios of the reagents in EtOH, the amount of the products was increased with 10% (*Scheme 6*).



Scheme 6

3.13. Similar transformation of 2-hydroxymethylene-5α-dihydrotestosterone (**21**), ammonium acetate and benzaldehyde (**24a**) in a molar ratio of 1:2:2 in EtOH under microwave irradiation afforded the ring A-fused pyrimidine derivative (**26a**) in a yield of 45%. After optimization of the reagent ratios (steroid:aldehyde:ammonium-acetate = 1:5:5), the yield of **26a** increased to 60% (*Scheme 7*).

3.14. The syntheses of similar 2'-arylpyrimidines were also carried out under the optimized conditions by using different substituted benzaldehyde derivatives (**24b–i**) in order to investigate the steric and electronic effects of the substituents of the reagents on the yields of the desired products (**26b–i**). The greater the distance of the group R from the reaction center was, the higher yield of the heterocyclic compound could be obtained. EWGs facilitated the reactions, while EDGs were less favorable for the ring-closure step compared to the reaction of benzaldehyde.



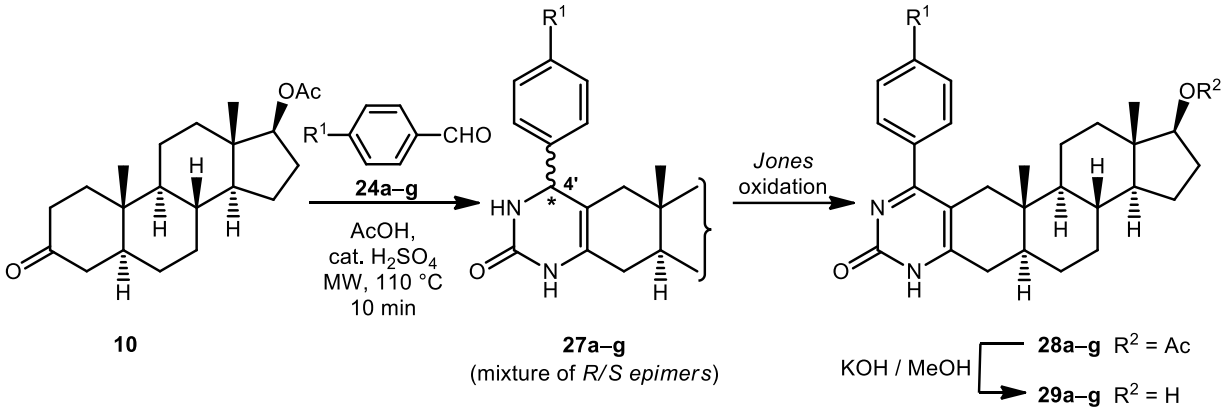
Aldehyde	R	Product	Yield (%)
24a	H	**26a**	60
24b	*p*-Cl	**26b**	62
24c	*p*-Br	**26c**	62
24d	*p*-F	**26d**	60
24e	*p*-NO_2	**26e**	69
24f	*p*-OMe	**26f**	44
24g	*p*-CH_3	**26g**	49
24h	*m*-CH_3	**26h**	43
24i	*o*-CH_3	**26i**	10

Scheme 7

3.15. The microwave-promoted *Biginelli*-type reaction of 17β-acetoxy-5α-dihydrotestosterone (**10**), benzaldehyde (**24a**), and urea in a molar ratio of 1:2:1 in acetic acid in the presence of a catalytic amount of cc. H_2SO_4 afforded an inseparable mixture of dihydropyrimidine diastereomers (**27a**-4'*R* and **27a**-4'*S*). The subsequent *Jones* oxidation led to a single pyrimidinone product (**28a**) (*Scheme 8*).

3.16. Reactions of **10,** *p*-substituted aromatic aldehydes (**24b–g**) and urea, applying similar conditions were also performed to investigate the substituent effect of the reagents to the cyclization process. We could not observe any substituent effect in the *Biginelli*-type reactions.

3.17. Deacetylation of the steroidal 2'-arylpyrimidinones (**28a–g)** was carried out in alkaline MeOH to furnish 17β-OH analogues (**29a–g)** in excellent yields.



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27a–g
(mixture of *R/S epimers*)

Aldehyde	R^1	Product	Yield (%)
24a	H	**28a**	67
24b	Cl	**28b**	62
24c	Br	**28c**	62
24d	F	**28d**	64
24e	NO_2	**28e**	69
24f	OMe	**28f**	60
24g	CH_3	**28g**	63

Scheme 8

4. Scientific publications forming the basis of the thesis

1. **Á. Baji**, A. Gyovai, J. Wölfling, R. Minorics, I. Ocsovszki, I. Zupkó, É. Frank
   Microwave-assisted one-pot synthesis of steroid–quinoline hybrids and an evaluation of their antiproliferative activities on gynecological cancer cell lines
   RSC Advances **2016**, *6*, 27501

   IF: 3,108

2. **Á. Baji**, F. Kovács, G. Mótyán, Gy. Schneider, J. Wölfling, I. Sinka, I. Zupkó, I. Ocsovszki, É. Frank
   Investigation of pH and substituent effects on the distribution ratio of novel steroidal ring D- and A-fused arylpyrazole regioisomers and evaluation of their cell-growth inhibitory effects *in vitro*
   Steroids, **2017**, *126*, 35.

   IF: 2,282*

3. **Á. Baji**, T. Kiss, J. Wölfling, D. Kovács, N. Igaz, M. K. Gopisetty, M. Kiricsi, É. Frank
   Multicomponent access to androstano-arylpyrimidines under microwave conditions and evaluation of their anti-cancer activity *in vitro*
   Journal of Steroid Biochemistry and Molecular Biology **2017**, *172*, 79.

   IF: 4,561*

Total IF: 9,951*

MTMT ID: 10053209

5. Scientific lectures and posters forming the basis of the thesis

Lectures:

1. **Á. Baji**, É. Frank
   Androsztánváz D-gyűrűjéhez kondenzált pirazolok előállítása mikrohullámú besugárzással
   2015.04.29., *Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 14. tudományos előadóülése,* Szeged

2. **Á. Baji**, É. Frank
   Szteránvázas heterociklusok előállítása multikomponensű reakciókkal
   2016.11.08., *A Magyar Tudomány Ünnepe – Új kihívások a gyógyszerkémiában*, Szeged

3. **Á. Baji**, É. Frank
   Szteránvázhoz kondenzált nitrogéntartalmú heterociklusok szintézise bifunkciós nemi hormon származékokból
   2017.11.27., *MTA Szteroid- és Terpenoidkémiai Munkabizottsági ülése,* Szeged.

Poster presentations:

1. **Á. Baji**, É. Frank, J. Wölfling, Gy. Schneider
   Efficient synthesis of steroidal ring A-fused quinolines by microwave irradiation
   2014.09.07–10., *22nd Conference on Isoprenoids,* Prague
   Chemicke Listy **2014**, *108*, s126.

2. **Á. Baji**, É. Frank, J. Wölfling, Gy. Schneider, I. Zupkó
   Microwave assisted synthesis of steroidal ring A-fused quinolines
   2015.06.16–19., *16th Tetrahedron Symposium*, Berlin (P2.058)

3. **Á. Baji**, É Frank, J. Wölfling
   Microwave-assisted synthesis of steroidal ring D-fused pyrazoles
   2015.03–05., *5th Meeting of the Paul Ehrlich MedChem Euro-PhD Network,* Cracow (P-2)

4. **Á. Baji**, G. Mótyán, É. Frank
   Pirazolgyűrűvel módosított androsztánvázas vegyületek mikrohullámú szintézise
   2015.08.31–09.02., *MKE 2. Nemzeti Konferencia*, Hajdúszoboszló (GY-P-2)

5. **Á. Baji**, É. Frank, M. Kiricsi
   Androsztánvázas pirimidinek szintézise és *in vitro* hatástani vizsgálata
   2017.06.19–21., *MKE 2017. évi Vegyészkonferencia*, Hajdúszoboszló (P-4)

6. **Á. Baji**, É. Frank, M. Kiricsi
   Multicomponent synthesis of novel androstano-pyrimidines and their pharmacological evaluation *in vitro*
   2017.06.25–28., *10th Joint Meeting on Medicinal Chemistry*, Dubrovnik (P-5)

7. É. Frank, **Á. Baji**, G. Mótyán, J. Wölfling, M. Kiricsi
   Ring A- or D-modified steroids with selective anticancer activity against androgen-independent prostate cancer cell lines
   2017.09.10–14., *42nd FEBS Congress*, Jerusalem (P-5.2.66)

6. Scientific publications forming the basis of the thesis

1. Z. Kádár, **Á. Baji**, I. Zupkó, T. Bartók, J. Wölfling, É. Frank É.
   Efficient approach to novel 1α-triazolyl-5α-androstane derivatives as potent antiproliferative agents
   Organic & Biomolecular Chemistry **2011**, *9*, 8051.

   IF: 3,696

2. G. Mótyán, **Á. Baji**, I. Zupkó, É. Frank
   Regio- and stereoselective synthesis of pregnane-fused isoxazolines by nitril-oxide/alkene 1,3-dipolar cycloaddition and an evaluation of their cell-growth inhibitory effect *in vitro*
   Journal of Molecular Structure **2016**, *1110*, 143.

   IF: 1,753

Total IF: 5,449

7. Scientific lectures and posters not forming the basis of the thesis

Lectures:

1. **Á. Baji**
   1α-Triazolil-5α-androsztán származékok előállítása intermolekuláris Cu(I)-katalizált cikloaddícióval
   2013.04.04–06., *XXXI. Országos Tudományos Diákköri Konferencia*, Eger

2. D. Kovács, J. Márton, **Á. Baji**, G. Mótyán, J. Wölfling, É. Frank
   Androsztánvázas 17-oxa-, és 17-tiadiazolok szintézise
   2013.11.12., *MTA Szteroid- és Terpenoidkémiai Munkabizottsági ülése*, Budapest

Poster presentations:

1. D. Kovács, G. Mótyán, **Á. Baji**, Gy. Schneider, É. Frank
   1,2,4-oxadiazolgyűrűvel módosított androsztánvázas vegyületek szintézise
   2013.06.26–28., *Vegyészkonferencia*, Hajdúszoboszló (P-31)

3. D. Kovács, G. Mótyán, **Á. Baji**, Gy. Schneider, É. Frank
   Efficient approach to steroidal 1,2,4-oxadiazoles in the androstane series
   2013.06.30–07.04., *VIIIth Joint Meeting on Medicinal Chemistry*, Lublin
   (P-23)

4. D. Kovács, G. Mótyán, **Á. Baji**, É. Frank, J. Wölfling
   Efficient approach to novel ring-condensed steroidal isoxazolines by 1,3-dipolar cycloaddition
   2014.06.29–07.04., *20th International Conference on Organic Synthesis*, Budapest (P-51)

5. **Á. Baji**, É. Frank, D. Kovács, L. Mérai
   Microwave-assisted access to novel steroidal 17β-3-pyrazole-5-ones
   2015.06.07–10., *IXth Joint Meeting in Medicinal Chemistry*, Athens (P-50)

6. B. Molnár, G. Mótyán, **Á. Baji**, É. Frank
   Pirazolin- és triazolgyűrűvel módosított ösztron származékok előállítása mikrohullámú aktiválással
   2017.06.19–21., *MKE Vegyészkonferencia*, Hajdúszoboszló (P-35)

7. G. Mótyán, **Á. Baji**, J. Wölfling, É. Frank, I. Zupkó
   Steroidal pyrazoles and pyrazolines as potent antfiproliferative agents
   2017.09.10–14., *42nd FEBS Congress*, Jerusalem (P-5.2.61)

Σ IF: 15,400